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Description

BACKGROUND OF THE INVENTION

This invention relates to a novel process for preparing 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds.

Optically active 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds are important compounds as intermediates of Diltiazem hydrochloride which is useful as a coronary vasodilating agent and other various pharmaceutical compounds.

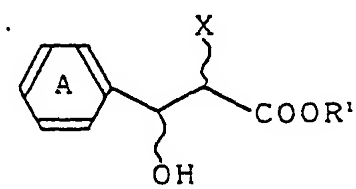
In the prior art, as the process for preparing 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds, there has been known the method in which a halogenoacetic acid ester compound and a benzaldehyde compound are permitted to react in the presence of an optically active lithium amide compound and alkyl lithium to give the above ester compounds (Japanese Unexamined Patent Publication No. 226881/1989).

However, this process employs an expensive optically active reagent to effect asymmetric induction, when reacting the halogenoacetatic acid ester compound and the benzaldehyde compound. Thus, there has been demanded a process for preparing 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds by using an enzyme in place of an expensive optically active reagent.

SUMMARY OF THE INVENTION

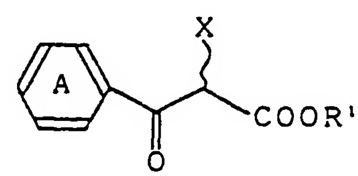
The present inventors have studied intensively, and consequently found that 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds can be obtained by permitting an enzyme having the ability of stereoselectively reducing oxo group to act on 2-halogeno-3-oxo-3-phenylpropionic acid ester compounds.

More specifically, according to the present invention, 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds represented by the formula (I):



(I)

wherein Ring A is a phenyl group which may be substituted, R^1 is an ester residue, and X is a halogen atom,
can be prepared by permitting an enzyme having the ability of stereoselectively reducing oxo group to hydroxy group to act on 2-halogeno-3-oxo-3-phenylpropionic acid ester compounds represented by the formula (II):



(II)

wherein Ring A, R^1 and X have the same meanings as defined above.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The process of the present invention can be practiced in the case when Ring A of the 2-halogeno-3-oxo-3-phenylpropionic acid ester compounds represented by the formula (II) is a phenyl group substituted, for example, with a lower alkyl group, a lower alkoxy group or a halogen atom as well as in the case when Ring A is unsubstituted phenyl group. The halogen atom represented by X is fluorine, chlorine, bromine or iodine atom. The ester residue represented by R^1 is generally a lower alkyl group, as exemplified by methyl

group, ethyl group, isopropyl group or t-butyl group. Ring A is preferably a phenyl group substituted with a lower alkyl group or a lower alkoxy group, and R^1 is preferably a lower alkyl group.

According to the process of the present invention, by selecting an enzyme to be used suitably, a carbonyl group at the 3-position of the starting material (II) can be reduced st reoselectively to a hydroxymethylene group having an absolute configuration of R to obtain (2R, 3R) type and/or (2S, 3R) type desired products, or reduced to a hydroxymethylene group having an absolute configuration of S to obtain (2R, 3S) type and/or (2S, 3S) type desired products.

Further, among the enzymes to be used in the present method, there exist those which can reduce only one isomer of the susbstrate having either R or S absolute configuration at 2-position thereof (namely, 2R- or 2S-isomer) and produce stereoselectively single isomer of the desired product having specified absolute configurations in both two asymmetric carbon atoms at 2- and 3-positions thereof (namely, either one of 2R,3R-, 2R,3S-, 2S,3R- or 2S,3S-isomer).

The enzyme to be used in the present method include reductase. Representative examples of the reductase may be those derived from microorganisms, which may be extracted from microbial cells according to the known method. A culture broth of a microorganism producing the above-mentioned enzyme, microbial cells collected from said culture broth and a processed product of said microbial cells can be used as the enzyme sources.

Such microorganisms include yeasts, bacteria, molds and actinomycetes. Examples of the yeasts may include microorganisms belonging to the genus *Candida*, the genus *Cryptococcus*, the genus *Hansenula*, the genus *Nematospora*, the genus *Rhodotorula*, the genus *Saccharomyces* and the genus *Zygosaccharomyces*; examples of the bacteria, microorganisms belonging to the genus *Arthrobacter*; examples of the molds, microorganisms belonging to the genus *Absidia*, the genus *Mucor* and the genus *Trichoderma*; and examples of the actinomycetes, microorganisms belonging to the genus *Mycobacterium* and the genus *Streptomyces*, respectively.

As specific examples of such microorganisms, there may be included, for example, *Candida maltosa* JCM 1504, Ditto IAM 12247, *Candida tropicalis* IFO 0589, Ditto IFO 1400, Ditto IFO 1647, Ditto IFO 1401, Ditto IFO 1404, *Cryptococcus laurentii* OUT 6027, *Hansenula anomala* IFO 0118, Ditto IFO 0149, *Hansenula minuta* IFO 0975, *Hansenula nonfermentans* IFO 1473, *Nematospora coryli* IFO 0658, *Rhodotorula glutinis* IFO 0389, *Zygosaccharomyces rouxii* IFO 1814, *Arthrobacter protophormiae* IFO 12128, *Absidia corymbifera* IFO 4009, *Mucor ambiguus* IFO 6742, *Mucor angulimacrosporus* IAM 6149, *Mucor circinelloides* IFO 6746, *Mucor fragilis* IFO 6449, *Mucor flavus* IAM 6143, *Mucor hiemalis* OUT 1045, Ditto IFO 6753, *Mucor janssenii* OUT 1050, *Mucor javanicus* IFO 4569, Ditto IFO 4570, Ditto IFO 4572, *Mucor racemosus* IFO 4581, *Trichoderma viride* OUT 4283, Ditto OUT 4289, Ditto OUT 4642, Ditto OUT 4644, Ditto IFO 5720, Ditto IFO 31137, *Mycobacterium smegmatis* IFO 3154, *Mycobacterium phlei* IFO 3158, *Streptomyces olivochromogenes* IFO 3178 and *Saccharomyces cerevisiae* (a baker's yeast, manufactured by Oriental Kobo Kogyo K.K.).

These may be either wild strains or mutant strains, and further may be those derived from these microorganisms according to the bioengineering methods such as gene recombination and cell fusion.

Further, the culture broth and cells of the above microorganisms can be cultured in a medium for said microorganisms generally used in this field of the art, for example, in a conventional medium containing carbon sources, nitrogen sources and inorganic salts, at room temperature or under heating (preferably about 20 to 40 °C) and also under aerobic conditions at pH 2 to 8. If necessary, the cells can be obtained by separating and collecting from the culture broth according to a conventional method.

The enzyme to be employed in the present method may be used in the form of lyophilized cells and acetone dried cells of the above-mentioned microorganisms. Further, the microbial cells or treated cells can be also immobilized by known methods such as the polyacrylamide method, the sulfur-containing polysaccharide gel method (e.g. carrageenan gel method), the alginic acid gel method or the agar gel method, before use.

The stereoselective reduction reaction of the present invention can be practiced by permitting the enzyme to act on 2-halogeno-3-oxo-3-phenylpropionic acid ester compounds (II).

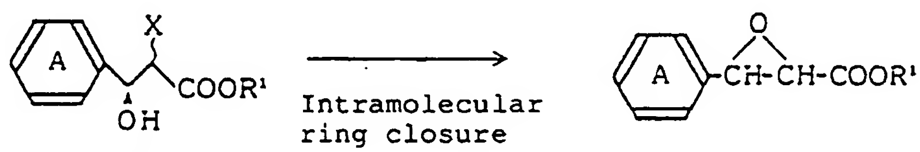
The substrate concentration may be generally 0.1 to 20 %, particularly preferably 0.2 to 10 %, and the reaction can proceed at normal temperature or under heating, preferably at 10 to 50 °C, particularly preferably at 25 to 40 °C. During the reaction, it is preferred to adjust the pH of the reaction mixture to 2 to 6, above all 3 to 5. In this case, since many substrates are difficultly soluble in water, a small amount of a water miscible organic solvent, for example, dimethylformamide or a lower alkanol such as methanol and ethanol may be used as a dissolving aid. Further, the reaction can be practiced in an aqueous solution, an organic solvent or a two-phase solvent of an aqueous solvent and an organic solvent. As such an organic solvent, there may be included water-immiscible organic solvent, for example, an aromatic solvent such as

benzene, toluene; an alkane such as n-hexane; ther solvent such as diethyl ether, diisopropyl ether and ester solvent such as ethyl acetate.

After completion of the reaction, the reaction mixture is extracted with an organic solvent such as toluene, chloroform and ethyl acetate, and the organic layer was condensed and applied to chromatography or distillation to obtain optically active 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds (I).

The 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds (I) thus obtained can be converted to a corresponding 3-phenylglycidates by intramolecular ring closure reaction in an appropriate solvent (e.g. a lower alkanol) under the presence of a base (e.g. an alkali metal alkoxide) at -10 to 50 °C, preferably 0 °C to room temperature.

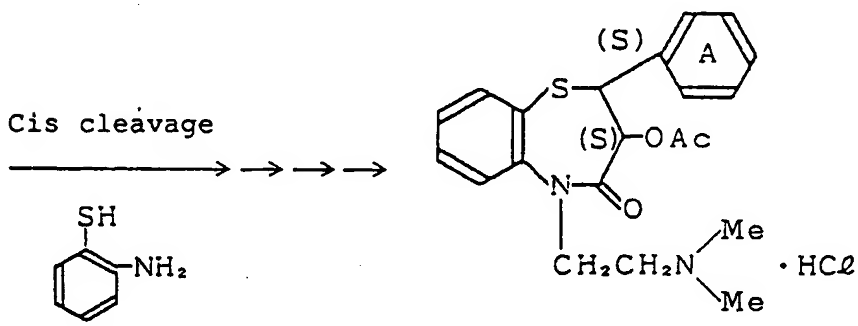
During the reaction, since the 3S type desired products, i.e. (2R, 3S) type and (2S, 3S) type desired products form a trans-(2R, 3S) type glycidate by intramolecular ring closure reaction regardless of the absolute configuration of the 2-position, the above products are used for synthesizing Diltiazem hydrochloride as shown below.



(3S) (I)

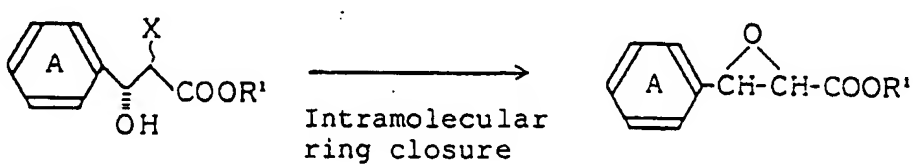
Trans-(2R, 3S) (III)

(Ring A = p-methoxyphenyl group)



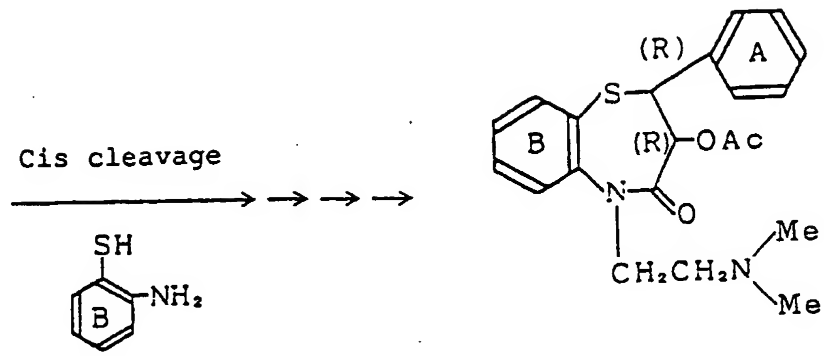
Diltiazem hydrochloride
(IV-1)

On the other hand, since the 3R type desired products, i.e. (2R, 3R) type and (2S, 3R) type desired products form a trans-(2S, 3R) type glycidate by intramolecular ring closure reaction regardless of the absolute configuration of the 2-position, the above products are used for synthesizing a benzothiazepine compound disclosed in, for example, Japanese Unexamined Patent Publication No. 202871/1985 as shown below.



(3R) (I)

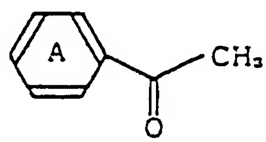
Trans-(2S, 3R) (III)



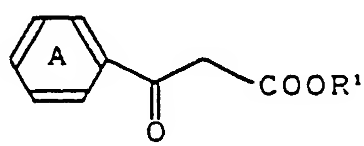
(IV-2)

In the above formulae, Ring A and Ring B each represent a benzene ring which may be substituted.

The 2-halogeno-3-oxo-3-phenylpropionic acid ester compounds (II) which are the starting material of the present invention can be prepared by reacting the acetophenone compound represented by the following formula:



wherein Ring A has the same meaning as defined above, with a di-lower alkyl carbonate in an appropriate solvent (e.g. ethers and aromatic hydrocarbons) under the presence of a base (e.g. an alkali metal and an alkali metal hydride) to prepare 3-oxo-3-phenylpropionic acid ester compounds represented by the following formula:



wherein Ring A and R^1 have the same meanings as defined above,
and then reacting the esters obtained with a halogenating agent (e.g. sulfuryl halide and N-halosuccinimide).

The above-mentioned process of the present invention can easily prepare (3R) or (3S) type 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds (I) without necessity of using an expensive optically active reagent and yet without complicated steps, and therefore, it can be an industrially advantageous preparation process.

EXAMPLES

The present invention is described below in detail by referring to Examples and Reference examples.

Example 1

Each 100 ml of a medium (adjusted pH to 6.5 with 0.1N-sodium hydroxide) containing 5 % of glucose, 0.1 % of potassium dihydrogen phosphate, 0.1 % of ammonium sulfate, 0.05 % of urea, 0.05 % of magnesium sulfate•heptahydrate, 0.05 % of calcium chloride•dihydrate, 0.1 % of yeast extract, 0.0002 % of ferrous sulfate•heptahydrate, 0.0002 % of manganese chloride•tetrahydrate and 0.0002 % of zinc sulfate•heptahydrate was charged in 29 shaking flasks having a volume of 500 ml, respectively, and

sterilized at 120 °C for 10 minutes. A platinum loop of *Mucor ambiguus* IFO 6742 was inoculated into 100 ml of the same media and the inoculated media was cultured at 30 °C for 72 hours. One ml of the culture broth was poured into each of the sterilized media contained in 29 flasks and the mixture were further shaken at 30 °C for 72 hours.

The cells collected by filtration with a sterilized gauze from the above culture broths were suspended in 100 ml of a McIlvaine buffer (pH 5.0) containing 5 % of glucose, respectively, and further to each shaking flask was added 0.2 ml of dimethylformamide containing 200 mg of methyl 2-chloro-3-oxo-3-(p-methoxyphenyl)propionate, followed by shaking reaction at 30 °C for 24 hours.

After these reaction mixtures were collected and 2,900 ml of ethyl acetate was added thereto, the cells were removed by filtration and an organic layer was separated, followed further by extraction of an aqueous layer again with 2,900 ml of ethyl acetate. The organic layers were mixed, washed with saturated saline solution, and dried. Subsequently, the solvent was evaporated under reduced pressure. Purification was carried out by silica gel chromatography (solvent: n-hexane/chloroform/ethyl acetate = 4/1/1) to obtain 2,320 mg of methyl (2S, 3R)-2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate.

The absolute configuration of the desired product isolated was determined according to the method in Reference example 1.

$[\alpha]_D^{23}$ +15.5° (c = 1.023, methanol)

NMR δ ($CDCl_3$):

2.89 (1H, d, J=3.9Hz, OH), 3.67, 3.80 (3Hx2, sx2, OCH_3, CO_2CH_3), 4.42 (1H, d, J=6.8Hz, -CHCl), 5.08 (1H, d of d, J=6.8Hz, 3.9Hz, -C<u>H</u>OH), 6.89 (2H, d, J=8.8Hz), 7.30 (2H, d, J=8.8Hz)

IR (Nujol) ν_{max} cm^{-1}:

3520, 1745, 1615, 1515, 1250, 1170, 1030, 825

MS(m/e): 244 (M^+)

m.p.: 103 to 103.5 °C

Optical purity syn *(2S, 3R) isomer: >99 % e.e.

<u>Example 2</u>

A platinum loop of *Nematospora coryli* IFO 0658 was inoculated in the same media as Example 1 in place of *Mucor ambiguus* and the inoculated media was cultured at 30 °C for 24 hours. 1 ml of the culture broth was poured into each of 29 flasks containing the same media as mentioned above and the mixtures were further shaken at 30 °C for 24 hours.

The cells were collected from the above culture broths, and then reaction, extraction and purification were carried out in the same manner as in Example 1 to obtain 300 mg of a mixture of methyl (2S, 3S) and (2R, 3S)-2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionates.

The absolute configuration was determined according to the method in Reference example 1.

NMR δ ($CDCl_3$): anti isomer:syn isomer = 1:2

Anti isomer: 2.89 (1H, d, J=4.6Hz, O<u>H</u>), 3.80 (6H, s, OC<u>H</u>$_3$, CO$_2$C<u>H</u>$_3$), 4.36 (1H, d, J=8.1Hz, -C<u>H</u>Cl), 5.00 (1H, d of d, J=8.1Hz, 4.6Hz, -C<u>H</u>OH), 6.91 (2H, d, J=8.8Hz), 7.32 (2H, d, J=8.8Hz)

Syn isomer: 2.89 (1H, d, J=3.9Hz, O<u>H</u>), 3.67, 3.80 (3Hx2, sx2, OC<u>H</u>$_3$, CO$_2$C<u>H</u>$_3$), 4.42 (1H, d, J=6.8Hz, -C<u>H</u>Cl), 5.08 (1H, d of d, J=6.8Hz, 3.9Hz, -C<u>H</u>OH), 6.89 (2H, d, J=8.8Hz), 7.30 (2H, d, J=8.8Hz)

IR (Nujol) ν_{max} cm^{-1}:

3520, 3450, 1745, 1730, 1615, 1515, 1250, 1170, 1030, 825

MS(m/e): 244 (M^+)

Optical purity anti (2S, 3S) isomer: 87 % e.e.

syn (2R, 3S) isomer: 96 % e.e.

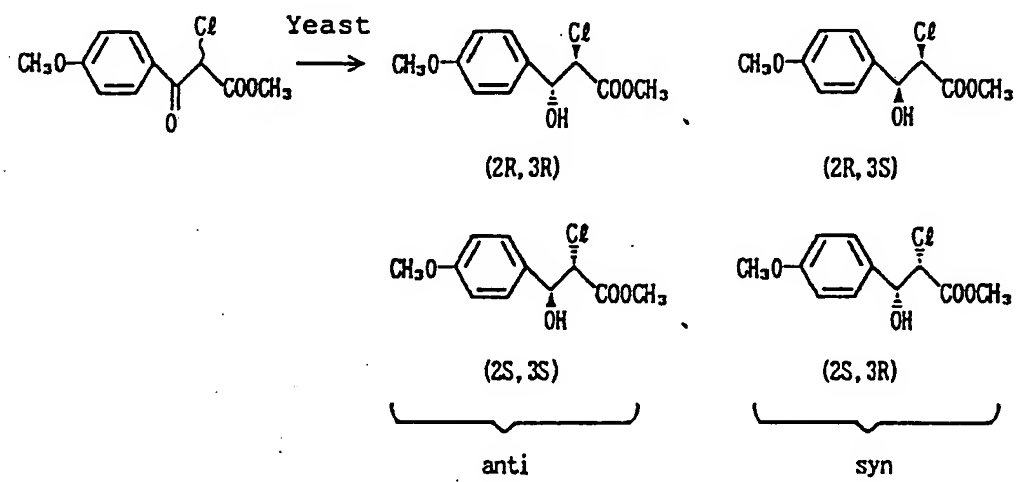
* In the present specification, syn and anti are indicated according to the definition by Masamune et al described in Angew. Chem. Int. Ed., Vol. 19, pp. 557 to 558 (1980).

Example 3

3 ml of the same medium (pH 6.5) as in Example 1 was charged into a test tube with an outer diameter of 15 mm, and sterilized at 120 °C for 10 minutes. Into the medium was inoculated a platinum loop of a yeast shown in Table 1 below, and the yeast was subjected to shaking culture at 30 °C for 24 hours. The cells collected by centrifugation from the above culture broth were suspended in 3 ml of a McIlvaine buffer (pH 5.0) containing 5 % of glucose, and 6 µl of dimethylformamide containing 6 mg of methyl 2-chloro-3-oxo-3-(p-methoxyphenyl)propionate was charged thereinto, followed by shaking reaction at 30 °C for 24 hours. Then, the reaction mixture was extracted with 2 ml of ethyl acetate and then the amount of methyl 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate formed was quantitated. Quantitation was performed by high performance liquid chromatography by use of a ZORBAX CN (trade name) 4.6 mmΦ x 250 mm manufactured by Du Pont Instruments and marketed by Shimadzu Corporation. The absolute configuration of the desired product isolated by thin layer chromatography (silica gel, solvent: n-hexane/chloroform/ethyl acetate = 4/1/1) was determined according to the method in Reference example 1.

In Table 1, the amount formed and optical purity thereof are shown.

Table 1 (Yeast)



Microorganism used	Methyl 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate		
	Amount formed (mg)	anti/syn	Optical purity (% e.e.)
Candida maltosa JCM 1504	0.8	77/23	anti (2R, 3R): 37 syn (2S, 3R): 75
Candida maltosa IAM 12247	0.6	73/27	anti (2R, 3R): 36 syn (2S, 3R): 53
Candida tropicalis IFO 0589	0.1	28/72	anti (2R, 3R): 12 syn (2S, 3R): 91
Candida tropicalis IFO 1400	0.1	84/16	anti (2R, 3R): >99 syn (2S, 3R): >99
Candida tropicalis IFO 1647	0.2	82/18	anti (2R, 3R): 35 syn (2S, 3R): 60
Candida tropicalis IFO 1401	0.3	72/28	anti (2R, 3R): 46 syn (2S, 3R): 90
Candida tropicalis IFO 1404	0.1	95/5	anti (2R, 3R): >99 syn (2S, 3R): 65
Cryptococcus laurentii OUT 6027	2.6	80/20	anti (2R, 3R): >99 syn (2S, 3R): 99
Hansenula anomala IFO 0118	0.2	86/14	anti (2R, 3R): 3 syn (2S, 3R): 78
Hansenula anomala IFO 0149	0.4	87/13	anti (2R, 3R): 63 syn (2S, 3R): >99
Hansenula minuta IFO 0975	1.5	63/37	anti (2R, 3R): 99 syn (2S, 3R): 97
Hansenula nonfermentans IFO 1473	0.8	47/53	anti (2R, 3R): >99 syn (2S, 3R): 98
Zygosaccharomyces rouxii IFO 1814	0.6	51/49	anti (2S, 3S): 91 syn (2R, 3S): 92
Rhodotorula glutinis IFO 0389	0.8	1/99	syn (2R, 3S): 96

Example 4

A medium (adjusted pH to 7.0 with 0.1N-sodium hydroxide) containing 1 % of glucose, 1 % of peptone, 0.1 % of potassium dihydrogen phosphate, 0.1 % of ammonium sulfate, 0.05 % of urea, 0.05 % of magnesium sulfate•heptahydrate, 0.05 % of calcium chloride•dihydrate, 0.1 % of yeast extract, 0.0002 % of ferrous sulfate•heptahydrate, 0.0002 % of manganese chloride•tetrahydrate and 0.0002 % of zinc sulfate•heptahydrate was charged into a test tube with an outer diameter of 15 mm, and sterilized at 120 °C for 10 minutes. In the same manner as in Example 3, a platinum loop of a bacterium shown in Table 2 below was inoculated, and the bacterium was subjected to shaking culture for 24 hours. Also in the same manner, reaction, extraction and quantitation were carried out.

In Tabl 2, the amount formed and optical purity thereof are shown.

Table 2 (Bacterium)

Microorganism used	Methyl 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate		
	Amount formed (mg)	anti/syn	Optical purity (% e.e.)
Arthrobacter protophormiae IFO 12128	0.1	>99/1	anti (2S, 3S): >99

Example 5

In the same manner as in Example 3, a platinum loop of a mold shown in Table 3 below was inoculated, and the mold was subjected to shaking culture for 72 hours. Also in the same manner, reaction, extraction and quantitation were carried out.

In Table 3, the amount formed and optical purity thereof are shown.

Table 3 (Mold)

Microorganism used	Methyl 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate		
	Amount formed (mg)	anti/syn	Optical purity (% e.e.)
Absidia corymbifera IFO 4009	1.1	1/>99	syn (2S, 3R): >99
Mucor angulimacrosporus IAM 6149	0.8	11/89	anti (2R, 3R): >99 syn (2S, 3R): >99
Mucor circinelloides IFO 6746	0.5	1/>99	syn (2S, 3R): >99
Mucor fragilis IFO 6449	0.2	1/>99	syn (2S, 3R): >99
Mucor flavus IAM 6143	0.3	15/85	anti (2R, 3R): 72 syn (2S, 3R): 98
Mucor hiemalis OUT 1045	1.2	12/88	anti (2R, 3R): 68 syn (2S, 3R): >99
Mucor janssenii OUT 1050	1.4	5/95	anti (2R, 3R): >99 syn (2S, 3R): >99
Mucor javanicus IFO 4569	1.7	7/93	anti (2R, 3R): >99 syn (2S, 3R): >99
Mucor javanicus IFO 4570	2.4	7/93	anti (2R, 3R): >99 syn (2S, 3R): >99
Mucor javanicus IFO 4572	0.2	1/>99	syn (2S, 3R): >99
Mucor racemosus IFO 4581	0.7	18/82	anti (2R, 3R): 36 syn (2S, 3R): 99
Mucor hiemalis IFO 6753	1.4	8/92	anti (2R, 3R): 27 syn (2S, 3R): >99
Trichoderma viride OUT 4283	2.2	38/62	anti (2S, 3S): 80 syn (2R, 3S): 94
Trichoderma viride OUT 4289	2.5	36/64	anti (2S, 3S): 85 syn (2R, 3S): 81
Trichoderma viride OUT 4642	3.5	44/56	anti (2S, 3S): 87 syn (2R, 3S): 96
Trichoderma viride OUT 4644	0.4	75/25	anti (2S, 3S): 97 syn (2R, 3S): 68
Trichoderma viride IFO 5720	2.5	41/59	anti (2S, 3S): 82 syn (2R, 3S): 91
Trichoderma viride IFO 31137	1.3	37/63	anti (2S, 3S): 78 syn (2R, 3S): 96

Example 6

By using a medium having the same composition as in Example 1 and having pH adjusted to 7.3 with 0.1N sodium hydroxide, in the same manner as in Example 3, a platinum loop of an actinomycete shown in

Table 4 below was inoculated, and the actinomycete was subjected to shaking culture at 30 °C for 72 hours. Also in the same manner, reaction, extraction and quantitation were carried out.

In Table 4, the amount formed and optical purity thereof are shown.

Table 4 (Actinomycete)

Microorganism used	Methyl 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate		
	Amount formed (mg)	anti/syn	Optical purity (% e.e.)
Mycobacterium smegmatis IFO 3154	0.1	82/18	anti (2R, 3R): 53 syn (2S, 3R): 22
Mycobacterium phlei IFO 3158	0.5	76/24	anti (2S, 3S): 56 syn (2R, 3S): 31
Streptomyces olivochromogenes IFO 3178	0.3	>99/1	anti (2S, 3S): 92

Example 7

In a test tube with an outer diameter of 15 mm, 0.25 g of a baker's yeast (*Saccharomyces cerevisiae*, manufactured by Oriental Kobo Kogyo K.K.) was suspended in 3 ml of a McIlvaine buffer containing 0.54 g of glucose dissolved therein, and then 6 µl of dimethylformamide containing 6 mg of methyl 2-chloro-3-oxo-3-(p-methoxyphenyl)propionate was charged, followed by shaking reaction at 30 °C for 24 hours. When the reaction mixture was extracted with 2 ml of ethyl acetate and the amount of methyl 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate formed was quantitated, it was found that 0.6 mg of the product was formed. In this case, the forming ratio of anti isomer to syn isomer and optical purity thereof are shown below.

Forming ratio: anti/syn = 62/38
Optical purity: anti (2R, 3R) isomer: >99 % e.e.
syn (2S, 3R) isomer: >99 % e.e.

Reference example 1

Condensation reaction between p-anisaldehyde and methyl chloroacetate was effected in tetrahydrofuran at -78 °C in the presence of lithium diisopropylamide, and the methyl 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate obtained was separated into anti isomer (a mixture of (2R, 3R) isomer and (2S, 3S) isomer) and syn isomer (a mixture of (2R, 3S) isomer and (2S, 3R) isomer) by silica gel chromatography (solvent: n-hexane/ethyl acetate = 3/1). NMR of these isomers was measured to determine anti isomer and syn isomer. When these isomers were analyzed by high performance liquid chromatography by use of a chiralcel OJ 4.6 mmΦ x 250 mm manufactured by Dicel Chemical Industries, LTD. (mobile phase: n-hexane/i-propanol = 7/3, flow rate: 1.0 ml/min, column temperature: 40 °C), a retention time of the anti isomer was 9.2 minutes and 9.9 minutes, and a retention time of the syn isomer was 11.7 minutes and 13.9 minutes.

The absolute configuration of the methyl 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate obtained in Example 1 was determined to be (2S, 3R) isomer since it had a retention time of 13.9 minutes, and it could be induced to methyl (2S, 3R)-3-(p-methoxyphenyl)glycidate by ring closure with sodium methylate in a methanol solution. Further, the methyl 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate obtained in Example 2 was applied to silica gel chromatography (solvent: n-hexane/chloroform/ethyl acetate = 4/1/1) to give mainly two compounds which have retention times of 9.9 and 11.7 minutes, respectively in the above high performance liquid chromatography. Both of these compounds could be induced to methyl (2R, 3S)-3-(p-methoxyphenyl)glycidate by ring closure with sodium methylate in a methanol solution.

Accordingly, the relationship between the retention time obtained by the above high performance liquid chromatography and the absolute configuration of methyl 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate was defined as follows.

(2R, 3R) isomer	9.2 minutes
(2S, 3S) isomer	9.9 minutes
(2R, 3S) isomer	11.7 minutes
(2S, 3R) isomer	13.9 minutes

Reference example 2

(1) 30.7 g of sodium was added to 300 ml of toluene, and the mixture was heated to 93 °C, followed by cooling while vigorous stirring, to give sandy products. To the product, 600 ml of toluene containing 300 g of dimethyl carbonate was added, and further 250 ml of a toluene solution containing 100 g of p-methoxyacetophenone was added dropwise over 2 hours and 15 minutes at 84 to 86 °C. After the dropwise addition, the mixture was stirred under heating at 82 to 83 °C for 1.5 hours, and then the solvent was evaporated. To the residue was added 1 ℓ of isopropyl ether, and crystals were collected by filtration and washed with 500 ml of isopropyl ether. These crystals were added to a mixture of ice and ethyl acetate containing 100 g of acetic acid, and extracted with ethyl acetate. The extract was washed with 5 % sodium hydrogen carbonate and then with water, and dried. Subsequently, the solvent was evaporated to obtain 131.6 g of methyl 3-oxo-3-(p-methoxyphenyl)propionate as an oily product.

$$\text{IR (liquid)}\ \nu_{max}\,\text{cm}^{-1}:$$

3625 to 3450, 1740, 1655

NMR δ ($CDCl_3$):

3.74 (3H, s), 3.87 (3H, s), 3.95 (2H, s), 6.94 (2H, d, J=9.2Hz), 7.92 (2H, d, J=9.2Hz)

(2) 131 g of the methyl 3-oxo-3-(p-methoxyphenyl)propionate obtained in the above (1) was dissolved in 1.3 ℓ of tetrachloromethane, and to the solution was added dropwise 85 g of sulfuryl chloride at 45 to 50 °C over 1 hour. After the dropwise addition, the mixture was stirred at the same temperature for 1 hour, cooled, washed with water and dried. Subsequently, the oily product of the residue obtained by evaporation of the solvent was distilled under reduced pressure to obtain 140 g of methyl 2-chloro-3-oxo-3-(p-methoxyphenyl)propionate.

b.p.: 143.5 to 145 °C/0.3 mmHg

$$\text{IR (liquid)}\ \nu_{max}\,\text{cm}^{-1}:$$

1750, 1660

NMR δ ($CDCl_3$):

3.82 (3H, s), 3.88 (3H, s), 5.91 (1H, s), 6.96 (2H, d, J=9.0Hz), 7.97 (2H, d, J=9.0Hz)

Reference example 3

(1) In a suspension containing 1.614 g of N,N'-dibenzoyl-L-cystine, 357 mg of t-butanol and 20 ml of tetrahydrofuran, 16 ml of a tetrahydrofuran solution containing 237 mg of lithium borohydride was added under argon atmosphere, and the mixture was refluxed for 1 hour. Subsequently, 10 ml of a tetrahydrofuran solution containing 728 mg of methyl 2-chloro-3-oxo-3-(p-methoxyphenyl)propionate was added at - 65 to -70 °C, and the mixture was stirred at the same temperature for 1 hour. After the reaction, the reaction mixture was decomposed with addition of 10 % hydrochloric acid, and then the pH was adjusted to 9 to 10 with addition of a 5 % aqueous sodium hydrogen carbonate solution, followed by extraction with ethyl ether. After the extract was washed with water and dried, the solvent was evaporated. 740 mg of the yellow oily product of the residue obtained was purified by silica gel column chromatography (solvent: hexane/ethyl acetate = 3/1) to obtain 660 mg of a mixture of syn (2R, 3S) isomer and anti (2S, 3S) isomer of methyl 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionate as a colorless crystal.

IR (liquid) $\nu_{max}^{cm^{-1}}$:

3480, 1750, 1610, 1515, 1250, 1175, 1030, 830

NMR δ ($CDCl_3$):

syn isomer: 2.89 (1H, d, 3.9Hz), 3.67 (3H, s), 3.80 (3H, s), 4.42 (1H, d, 6.8 Hz), 5.08 (1H, dd, 3.9 and 6.8Hz), 6.89 (2H, d, 8.8Hz), 7.30 (2H, d, 8.8Hz)

anti isomer: 2.89 (1H, d, 4.6Hz), 3.80 (6H, s), 4.35 (1H, d, 8.1Hz), 5.00 (1H, dd, 4.6 and 8.1Hz), 6.91 (2H, d, 8.8Hz), 7.32 (2H, d, 8.8Hz)

(2) To a solution containing the crystals obtained in the above (1) dissolved in 16 ml of methanol was added a methanol solution containing 153 mg of sodium methylate at 0 °C, and the mixture was stirred at the same temperature for 90 minutes, followed by stirring at room temperature for 10 minutes. Subsequently, water was added to the reaction mixture, and extraction was effected with ethyl ether. After the extract was washed with saturated saline solution and dried, the solvent was evaporated. The oily product of the residue obtained was purified by silica gel column chromatography (solvent: hexane/ethyl acetate = 3/1) to obtain 506 mg of methyl (2R, 3S)-3-(p-methoxyphenyl)glycidate.

$[\alpha]_D^{20}$ -143.5° (c = 0.30, methanol)

(Optical purity: 82 % e.e.; by HPLC)

IR (Nujol) $\nu_{max}^{cm^{-1}}$:

2920, 1730, 1615, 1520, 1440, 1250, 1030, 840

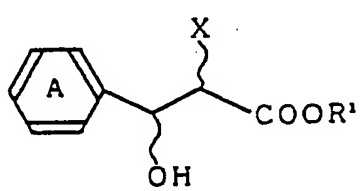
NMR δ ($CDCl_3$):

3.50 (1H, d, J=1.8Hz), 3.80 (3H, s), 3.81 (3H, s), 4.04 (1H, d, J=1.8Hz), 6.87 (2H, d, J=9.0Hz), 7.20 (2H, d, J=9.0Hz)

Mass (m/e): 208 (M^+)

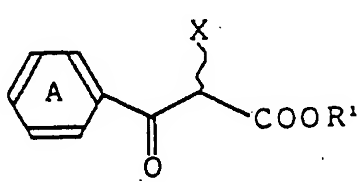
Claims

1. A process for preparing 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds represented by the formula (I):



(I)

wherein Ring A is a phenyl group which may be substituted, R^1 is an ester residue, and X is a halogen atom,
which comprises permitting an enzyme having the ability of stereoselectively reducing oxo group to hydroxy group to act on 2-halogeno-3-oxo-3-phenylpropionic acid ester compounds represented by the formula (II):



(II)

wherein Ring A, R^1 and X have the same meanings as defined above.

2. The process according to Claim 1, wherein the enzyme is a reductase.

3. The process according to Claim 2, wherein the reductase is derived from the microorganisms belonging to the genus selected from the group consisting of the genus *Candida*, the genus *Cryptococcus*, the genus *Hansenula*, the genus *Nematospora*, the genus *Rhodotorula*, the genus *Saccharomyces*, the genus *Zygosaccharomyces*, the genus *Arthrobacter*, the genus *Absidia*, the genus *Mucor*, the genus *Trichoderma*, the genus *Mycobacterium* and the genus *Streptomyces*.

4. The process according to either one of Claims 1 - 3, wherein the 3-position is reduced to an absolute configuration of R.

5. The process according to either one of Claims 1 - 3, wherein the 3-position is reduced to an absolute configuration of S.

6. The process according to either one of Claims 1 - 5, wherein Ring A is a lower alkylphenyl group or a lower alkoxyphenyl group, and R^1 is a lower alkyl group.

7. The process according to Claim 6, wherein Ring A is a lower alkoxyphenyl group.

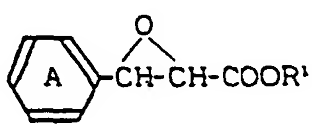
8. The process according to Claim 7, wherein the 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds are lower alkyl esters of 2-chloro-3-hydroxy-3-(p-methoxyphenyl)propionic acid.

9. The process according to either one of Claims 1 - 8, wherein said stereoselective reduction is carried out in a substrate concentration of 0.1 to 20 % at normal temperature or under heating and at a pH of 2 to 6.

10. The process according to Claim 9, wherein said reduction is carried out in a substrate concentration of 0.2 to 10 % at a temperature of 10 to 50 °C and at a pH of 3 to 5.

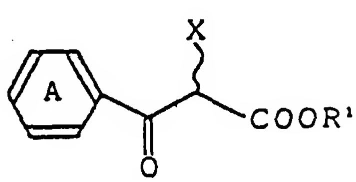
11. The process according to either one of Claims 1 - 10, wherein the reaction is carried out in an aqueous solution, an organic solvent or a two-phase solvent system of aqueous solution and organic solvent.

12. A process for preparing trans-3-phenylglycidates represented by the formula (III):



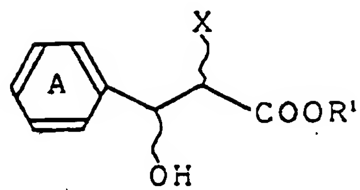
(III)

wherein Ring A is a phenyl group which may be substituted, R^1 is an ester residue, which comprises permitting an enzyme having the ability of stereoselectively reducing oxo group to hydroxy group to act on 2-halogeno-3-oxo-3-phenylpropionic acid ester compounds represented by the formula (II):



(II)

wherein X is a halogen atom, and Ring A and R^1 are the same meanings as defined above, to give 2-halogeno-3-hydroxy-3-phenylpropionic acid ester compounds represented by the formula (I):



(I)

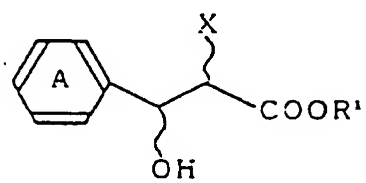
wherein Ring A, R^1 and X are the same meanings as defined above, and subjecting the thus-obtained compounds (I) to intramolecular ring closure reaction.

13. The process according to Claim 12, wherein Ring A is a lower alkylphenyl group or a lower alkoxyphenyl group, and R^1 is a lower alkyl group.

14. The process according to Claim 13, wherein Ring A is a lower alkoxyphenyl group.

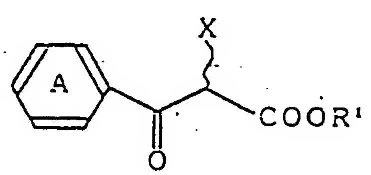
Patentansprüche

1. Verfahren zur Herstellung von 2-Halogen-3-hydroxy-3-phenylpropionsäureesterverbindungen, dargestellt durch die Formel (I):



(I)

worin der Ring A eine Phenylgruppe, die substituiert sein kann, bedeutet, R^1 einen Esterrest bedeutet und X ein Halogenatom bedeutet,
durch Einwirkung eines Enzyms, das die Fähigkeit besitzt, die Oxogruppe stereoselektiv in eine Hydroxygruppe zu reduzieren, auf 2-Halogen-3-oxo-3-phenylpropionsäureesterverbindungen, die durch die Formel (II):



(II)

dargestellt werden, worin der Ring A, R^1 und X die gleichen Bedeutungen, wie oben definiert, besitzen.

2. Verfahren nach Anspruch 1, dadurch **gekennzeichnet,** daß das Enzym eine Reduktase ist.

3. Verfahren nach Anspruch 2, dadurch **gekennzeichnet,** daß sich die Reduktase von Mikroorganismen ableitet, die zum Genus, ausgewählt aus der Gruppe Genus Candida, Genus Cryptococcus, Genus Hansenula, Genus Nematospora, Genus Rhodotorula, Genus Saccharomyces, Genus Zygosaccharomyces, Genus Arthrobacter, Genus Absidia, Genus Mucor, Genus Trichoderma, Genus Mycobacterium und Genus Streptomyces, gehören.

4. Verfahren nach einem der Ansprüche 1 bis 3, dadurch **gekennzeichnet,** daß die 3-Stellung zu einer absoluten Konfiguration von R reduziert worden ist.

5. Verfahren nach einem der Ansprüche 1 bis 3, dadurch **gekennzeichnet**, daß die 3-Stellung zu einer absoluten Konfiguration von S reduziert worden ist.

6. Verfahren nach einem der Ansprüche 1 bis 5, dadurch **gekennzeichnet**, daß der Ring A eine niedrige Alkylphenylgruppe oder eine niedrige Alkoxyphenylgruppe ist und R^1 eine niedrige Alkylgruppe bedeutet.

7. Verfahren nach Anspruch 6, dadurch **gekennzeichnet**, daß der Ring A eine niedrige Alkoxyphenylgruppe ist.

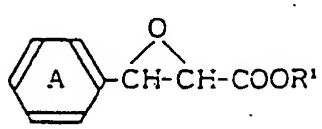
8. Verfahren nach Anspruch 7, dadurch **gekennzeichnet**, daß die 2-Halogen-3-hydroxy-3-phenylpropionsäureesterverbindungen niedrige Alkylester von 2-Chlor-3-hydroxy-3-(p-methoxyphenyl)-propionsäure sind.

9. Verfahren nach einem der Ansprüche 1 bis 8, dadurch **gekennzeichnet**, daß die stereoselektive Reduktion in einer Substratkonzentration von 0,1 bis 20 % bei Normaltemperatur oder unter Erhitzen und einem pH von 2 bis 6 durchgeführt wird.

10. Verfahren nach Anspruch 9, dadurch **gekennzeichnet**, daß die Reduktion in einer Substratkonzentration von 0,2 bis 10 % bei einer Temperatur von 10 bis 50 °C und einem pH von 3 bis 5 durchgeführt wird.

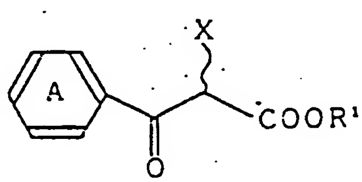
11. Verfahren nach einem der Ansprüche 1 bis 10, dadurch **gekennzeichnet**, daß die Reaktion in einer wäßrigen Lösung aus einem organischen Lösungsmittel oder einem zweiphasigen Lösungsmittelsystem aus wäßriger Lösung und organischem Lösungsmittel durchgeführt wird.

12. Verfahren zur Herstellung von trans-3-Phenylglycidaten, dargestellt durch die Formel (III):



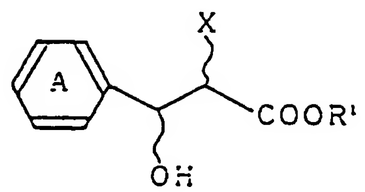
(III)

worin der Ring A eine Phenylgruppe, die substituiert sein kann, bedeutet, R^1 einen Esterrest bedeutet,
durch Einwirkung eines Enzyms, das die Fähigkeit besitzt, eine Oxogruppe in eine Hydroxygruppe stereoselektiv zu reduzieren, auf 2-Halogen-3-oxo-3-phenylpropionsäureesterverbindungen, dargestellt durch die Formel (II):



(II)

worin X ein Halogenatom bedeutet und die Ringe A und R^1 die gleiche Bedeutung wie oben besitzen,
wobei 2-Halogen-3-hydroxy-3-phenylpropionsäureesterverbindungen, dargestellt durch die Formel (I):



(I)

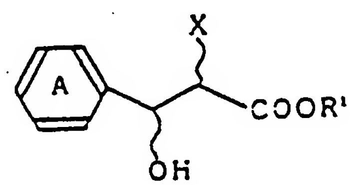
worin der Ring A, R[1] und X die gleiche Bedeutung, wie oben definiert, besitzen, gebildet werden, und die so erhaltenen Verbindungen (I) der intramolekularen Ringschlußreaktion unterworfen werden.

13. Verfahren nach Anspruch 12, dadurch **gekennzeichnet,** daß der Ring A eine niedrige Alkylphenylgruppe oder eine niedrige Alkoxyphenylgruppe bedeutet und R[1] eine niedrige Alkylgruppe bedeutet.

14. Verfahren nach Anspruch 13, dadurch **gekennzeichnet,** daß der Ring A eine niedrige Alkoxyphenylgruppe bedeutet.

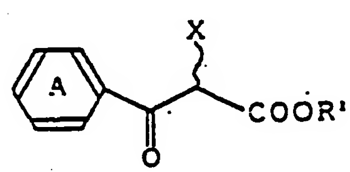
Revendications

1. Procédé pour préparer des composés esters d'acide 2-halogéno-3-hydroxy-3-phénylpropionique représentés par la formule (I) :



(I)

où le cycle A est un groupe phényle qui peut être substitué, R[1] est un résidu ester, et X est un atome d'halogène,
qui comprend l'étape qui consiste à laisser une enzyme ayant la capacité de réduire stéréosélectivement un groupe oxo en groupe hydroxy agir sur des composés esters d'acide 2-halogéno-3-oxo-3-phénylpropionique représentés par la formule (II) :



(II)

où le cycle A, R[1] et X ont les mêmes significations que celles définies ci-dessus.

2. Procédé selon la revendication 1, dans lequel l'enzyme est une réductase.

3. Procédé selon la revendication 2, dans lequel la réductase est obtenue à partir des micro-organismes appartenant à un genre choisi dans le groupe constitué du genre *Candida,* du genre *Cryptococcus*, du genre *Hansenula*, du genre *Nematospora*, du genre *Rhodotorula*, du genre *Saccharomyces*, du genre *Zygosaccharomyces*, du genre *Arthrobacter*, du genre *Absidia*, du genre *Mucor*, du genre *Trichoderma*, du genre *Mycobacterium* et du genre *Streptomyces*.

4. Procédé selon l'une quelconque des revendications 1 à 3, dans lequel la position 3 est réduite à une configuration absolue de R.

5. Procédé selon l'une quelconque des revendications 1 à 3, dans lequel la position 3 est réduite à une configuration absolue de S.

6. Procédé selon l'une quelconque des revendications 1 à 5, dans lequel le cycle A st un groupe alkylphényle inférieur ou un groupe alcoxyphényle inférieur, et R' est un group alkyle inférieur.

7. Procédé selon la revendication 6, dans lequel le cycle A est un groupe alcoxyphényle inférieur.

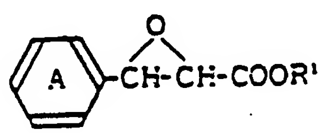
8. Procédé selon la revendication 7, dans lequel les composés esters d'acide 2-halogéno-3-hydroxy-3-phénylpropionique sont des esters d'alkyle inférieur de l'acide 2-chloro-3-hydroxy-3-(p-méthoxyphényl)-propionique.

9. Procédé selon l'une quelconque des revendications 1 à 8, dans lequel ladite réduction stéréosélective est effectuée dans une concentration de substrat de 0,1 à 20 % à une température normale ou en chauffant et à un pH de 2 à 6.

10. Procédé selon la revendication 9, dans lequel ladite réduction est effectuée dans une concentration de substrat de 0,2 à 10 % à une température de 10 à 50 °C et à un pH de 3 à 5.

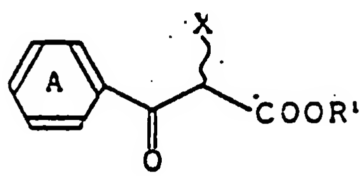
11. Procédé selon l'une quelconque des revendications 1 à 10, dans lequel la réaction est effectuée dans une solution aqueuse, un solvant organique ou un système solvant à deux phases d'une solution aqueuse et d'un solvant organique.

12. Procédé pour préparer des glycidates de trans-3-phényle représentés par la formule (III) :



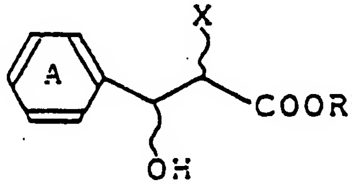
(III)

où le cycle A est un groupe phényle qui peut être substitué, R' est un résidu ester,
qui comprend l'étape qui consiste à laisser une enzyme ayant la capacité de réduire stéréosélectivement un groupe oxo en groupe hydroxy agir sur des composés esters d'acide 2-halogéno-3-oxo-3-phénylpropionique représentés par la formule (II) :



(II)

où X est un atome d'halogène, le cycle A et R' ont les mêmes significations que celles définies ci-dessus,
pour donner les composés esters d'acide 2-halogéno-3-hydroxy-3-phénylpropionique représentés par la formule (I) :



(I)

où le cycle A, R^1 et X ont les mêmes significations que celles définies ci-dessus,
et à soumettre les composés (I) ainsi obtenus à une réaction de fermeture du cycle intramoléculaire.

13. Procédé selon la revendication 12, dans lequel le cycle A est un groupe alkylphényle inférieur ou un groupe alcoxyphényle inférieur, et R^1 est un groupe alkyle inférieur.

14. Procédé selon la revendication 13, dans lequel le cycle A est un groupe alcoxyphényle inférieur.